# Guide to Pipetting Challenging Liquids

CORNING

Most commonly used pipettors (e.g., Corning® Lambda™ Plus Pipettor) work based on an air-cushion principle and are perfect for most pipetting applications. However, the precision and accuracy of the pipetting results can be affected when working with liquids of different temperatures or with a different viscosity, volatility, or density than water.

In these cases, positive-displacement pipettors (e.g., Corning Step-R™ Repeating Pipettor) can improve the pipetting process and subsequent results because there is no air-cushion and no variation of the volume aspirated in response to the physical properties of samples.
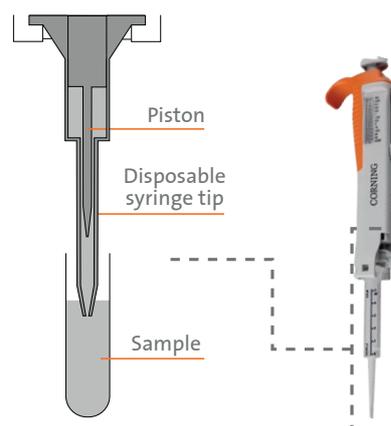
Air-displacement System
Corning Lambda Plus Pipettor

Piston
Shaft
Air cushion
Disposable pipet tip
Sample
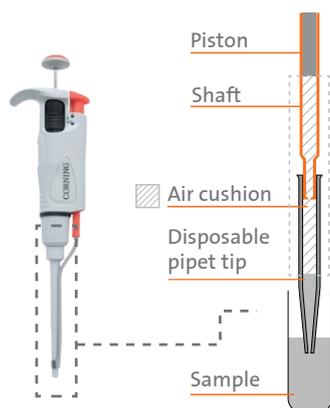
Positive-displacement System
Corning Step-R Repeating Pipettor

Piston
Disposable syringe tip
Sample

DENSITY

Problem: Pipetted volume is smaller than expected

Example: Chloroform

Cause: Higher density liquids have greater mass per unit volume, and impose an increased gravitational force on the air space between the liquid and the piston. The increased air space results in a smaller volume of liquid being aspirated into the tip. The liquid's density influences the size of the air cushion.

Solution:
‣ Pipettor recalibration when air-displacement system is used
‣ Positive-displacement system

VISCOSITY

Problem: Inaccurate dispenses; reduced liquid volume

Example: Glycerol, Corning Matrigel® Matrix

Cause: If pipetting is too fast during aspiration, air bubbles are often formed within the aspirated liquid. Viscous liquids tend to stick to the surface of the tip, and some portion of the pipetted liquid may remain in the pipet tip. The larger the air cushion (e.g., 100 µL dispensed using a 1000 µL tip), the greater the error. The greater the dispenser tip, the lower the dispensable viscosity.

Solution:
‣ Reverse pipetting when an air-displacement pipettor is used
‣ Pipetting slowly when an air-displacement pipettor is used
‣ Using wide bore orifice tips
‣ Positive-displacement system

HIGH VAPOR PRESSURE

Problem: Droplet hanging from the tip; the pipettor drips

Examples: Acetone, acetonitrile

Cause: Vapor pressure is a property that describes how fast liquid evaporates into the atmosphere until it reaches equilibrium. All liquids exist in equilibrium between their liquid and gas states. In finding this balance, a liquid will continue to evaporate until a certain concentration is present in its surrounding atmosphere. As soon as the liquid is aspirated into a pipettor tip, evaporation begins. The resulting pressure inside the pipettor begins to build and eventually forces some of the liquid back through the tip orifice. Liquids evaporate so quickly that they increase the internal pressure of the air-displacement tips, leading to leaks of the sample. Pre-wetting the tip will help the air space in the tip to reach a concentration closer to the equilibrium point.

Solution:
‣ Pre-wetting at least 5 times when an air-displacement pipettor is used
‣ Positive-displacement system

TEMPERATURE

Problem: Pipetted volume is smaller or larger than expected

Examples: Reagents and buffers at 37°C; nucleic acid-based reagents at 4°C or lower

Cause: When a tip is inserted into a warm liquid, the air in the tip is at ambient temperature. During aspiration, the tip heats up which causes the air to expand and push liquid out of the tip. This results in less liquid being delivered than expected. With cold liquids, it has the opposite effect.

Solution:
‣ Pre-wetting at least 5 times when an air-displacement pipettor is used.
‣ Use pipettors at, or close to, the nominal volume settings (this results in better accuracy compared to using the pipettors at, or close to, the minimum specified volume setting)

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